

WHAT IS CLAIMED IS:

- 1 **1.** A nucleic acid-lipid particle composition for introducing a nucleic acid
2 into a cell, said particle comprising: a cationic lipid, a conjugated lipid that inhibits
3 aggregation of particles, a nucleic acid and an endosomal membrane destabilizer.
- 1 **2.** The nucleic acid-lipid particle composition of claim **1**, wherein said
2 endosomal membrane destabilizer is outside said nucleic acid-lipid particle.
- 1 **3.** The nucleic acid-lipid particle composition of claim **1**, wherein said
2 endosomal membrane destabilizer is both outside and inside said nucleic acid-lipid particle.
- 1 **4.** The nucleic acid-lipid particle composition of claim **1**, wherein said
2 endosomal membrane destabilizer is Ca^{++} ion.
- 1 **5.** The nucleic acid-lipid particle composition of claim **4**, wherein the
2 concentration of Ca^{++} ion is from about 0.1 mM to about 100 mM.
- 1 **6.** The nucleic acid-lipid particle composition of claim **5**, wherein the
2 concentration of Ca^{++} ion is from about 1 mM to about 20 mM.
- 1 **7.** The nucleic acid-lipid particle composition of claim **1**, wherein said
2 particle has a median diameter of less than about 150 nm.
- 1 **8.** The nucleic acid-lipid particle composition of claim **1**, wherein said
2 cationic lipid is a member selected from the group consisting of N,N-dioleyl-N,N-
3 dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium bromide
4 (DDAB), N-(1-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP), N-
5 (1-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA), and N,N-
6 dimethyl-2,3-dioleyloxy)propylamine (DODMA), and combinations thereof.
- 1 **9.** The nucleic acid-lipid particle composition of claim **1**, wherein said
2 particle further comprises an additional noncationic lipid.
- 1 **10.** The nucleic acid-lipid particle composition of claim **9**, wherein said
2 noncationic lipid is selected from the group consisting of DOPE, POPC, and EPC.

1 **11.** The nucleic acid-lipid particle composition of claim 1, wherein said
2 particle comprises a functional group that facilitates Ca^{++} ion chelation.

1 **12.** The nucleic acid-lipid particle composition of claim 1, wherein said
2 conjugated lipid that inhibits aggregation of particles has the formula



4 wherein: A is a lipid moiety;
5 W is a hydrophilic polymer; and
6 Y is a polycationic moiety.

1 **13.** The nucleic acid-lipid particle composition of claim **12**, wherein W is a
2 polymer selected from the group consisting of PEG, polyamide, polylactic acid, polyglycolic
3 acid, polylactic acid/polyglycolic acid copolymers and combinations thereof, said polymer
4 having a molecular weight of about 250 to about 7000 daltons.

14. The nucleic acid-lipid particle composition of claim 12, wherein Y has
at least 4 positive charges at a selected pH.

1 15. The nucleic acid-lipid particle composition of claim 12, wherein Y is a
2 member selected from the group consisting of lysine, arginine, asparagine, glutamine,
3 derivatives thereof and combinations thereof.

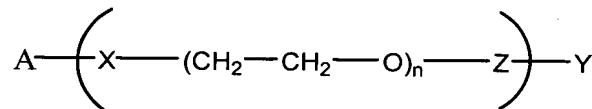
16. The nucleic acid-lipid particle composition of claim 12, wherein A is a
member selected from the group consisting of a diacylglycerolyl moiety, a dialkylglycerolyl
moiety, a N-N-dialkylamino moiety, a 1,2-diacyloxy-3-aminopropane moiety and a 1,2-
dialkyl-3-aminopropane moiety.

1 17. The nucleic acid-lipid particle composition of claim 12, wherein W is
2 PEG.

1 **18.** The nucleic acid-lipid particle composition of claim **12**, wherein W is a
2 polyamide polymer.

1 **19.** The nucleic acid-lipid particle composition of claim **12**, wherein W has
2 a molecular weight of about 250 to about 2000 daltons.

1 **20.** The nucleic acid-lipid particle composition of claim 17, having the
2 general structure of Formula II:



3 **II**

4 wherein

5 X is a member selected from the group consisting of a single bond or a
6 functional group covalently attaching said lipid to at least one ethylene oxide unit;

7 Z is a member selected from the group consisting of a single bond or a
8 functional group covalently attaching said at least one ethylene oxide unit to a cationic group;
9 and

10 n is an integer having a value of between about 6 to about 50.

1 **21.** The nucleic acid-lipid particle composition of claim 20, wherein

2 X is a member selected from the group consisting of a single bond,
3 phosphatidylethanolamino, phosphatidylethanolamido, phosphoro, phospho,
4 phosphoethanolamino, phosphoethanolamido, carbonyl, carbamate, carboxyl, carbonate,
5 amido, thioamido, oxygen, sulfur and NR, wherein R is a hydrogen or alkyl group.

1 **22.** The nucleic acid-lipid particle composition of claim 20, wherein

2 Z is a member selected from the group consisting of a single bond,
3 phosphatidylethanolamino, phosphatidylethanolamido, phosphoro, phospho,
4 phosphoethanolamino, phosphoethanolamido, carbonyl, carbamate, carboxyl, carbonate,
5 amido, thioamido, oxygen, sulfur and NR, wherein R is a hydrogen or alkyl group.

1 **23.** The nucleic acid-lipid particle composition of claim 20, wherein

2 A is a diacylglycerolyl moiety;

3 X is phosphoethanolamido;

4 Z is NR, wherein R is a hydrogen atom; and

5 Y is a member selected from the group consisting of about 1 to about 10 basic
6 amino acids or derivatives thereof.

1 **24.** The nucleic acid-lipid particle composition of claim 23, wherein

2 A is a diacylgercerolyl moiety having 2 fatty acyl chains, wherein each acyl
3 chain is independently between 2 and 30 carbons in length and is either saturated or has
4 varying degrees of saturation.

1 25. The nucleic acid-lipid particle composition of claim 23, wherein
2 Y is a member selected from the group consisting of lysine, arginine,
3 asparagine, glutamine, derivatives thereof and combinations thereof.

1 26. The nucleic acid-lipid particle composition of claim 23, wherein
2 A is a diacylgercerolyl moiety having 2 fatty acyl chains, wherein each acyl
3 chain is a saturated C-18 carbon chain; and
4 Y is a cationic group having 4 lysine residues or derivatives thereof.

1 27. The nucleic acid-lipid particle composition of claim 1, wherein said
2 conjugated lipid that inhibits aggregation of particles is a PEG-lipid.

1 28. The nucleic acid-lipid particle composition of claim 27, wherein said
2 PEG-lipid is PEG-ceramide.

1 29. The nucleic acid-lipid particle composition of claim 28, wherein the
2 ceramide of said PEG-ceramide comprises a fatty acid group having about 8 to about 20
3 carbon atoms.

1 30. The nucleic acid-lipid particle composition of claim 28, wherein said
2 PEG-lipid is PEG-phosphatidylethanolamine.

1 31. The nucleic acid-lipid particle composition of claim 1, wherein said
2 conjugated lipid that inhibits aggregation of particles is an ATTA-lipid.

1 32. The nucleic acid-lipid particle composition of claim 1, wherein said
2 nucleic acid is selected from the group consisting of a plasmid, an antisense oligonucleotide,
3 and a ribozyme.

1 33. A method of introducing a nucleic acid into a cell, said method
2 comprising:
3 contacting said cell with a nucleic acid-lipid particle composition, said particle
4 comprising a cationic lipid, a conjugated lipid that inhibits aggregation of particles, and a
5 nucleic acid; and an endosomal membrane destabilizer.

1 **34.** The method of introducing a nucleic acid into a cell of claim 33,
2 wherein said endosomal membrane destabilizer is outside said nucleic acid-lipid particle.

1 **35.** The method of introducing a nucleic acid into a cell of claim 33,
2 wherein said endosomal membrane destabilizer is Ca^{++} ion.

1 **36.** The method of introducing a nucleic acid into a cell of claim 35,
2 wherein the concentration of Ca^{++} ion is from about 0.1 mM to about 100 mM.

1 **37.** The method of introducing a nucleic acid into a cell of claim 36,
2 wherein the concentration of Ca^{++} ion is from about 1 mM to about 20 mM.

1 **38.** The method of introducing a nucleic acid into a cell of claim 33,
2 wherein said particle has a median diameter of less than about 150 nm.

1 **39.** The method of introducing a nucleic acid into a cell of claim 33,
2 wherein said cationic lipid is a member selected from the group consisting of N,N-dioleyl-
3 N,N-dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium
4 bromide (DDAB), N-(1-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride
5 (DOTAP), N-(1-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA),
6 and N,N-dimethyl-2,3-dioleyloxy)propylamine (DODMA), and combinations thereof.

1 **40.** The method of introducing a nucleic acid into a cell of claim 33,
2 wherein said particle further comprises an additional noncationic lipid.

1 **41.** The method of introducing a nucleic acid into a cell of claim 40,
2 wherein said noncationic lipid is selected from the group consisting of DOPE, POPC, and
3 EPC.

1 **42.** The method of introducing a nucleic acid into a cell of claim 33,
2 wherein said particle comprises a functional group that facilitates Ca^{++} ion chelation.

1 **43.** The method of introducing a nucleic acid into a cell of claim 33,
2 wherein said conjugated lipid that inhibits aggregation of particles has the formula



4 wherein: A is a lipid moiety;
5 W is a hydrophilic polymer; and
6 Y is a polycationic moiety.

1 **44.** The method of introducing a nucleic acid into a cell of claim 43,
2 wherein W is a polymer selected from the group consisting of PEG, polyamide, polylactic
3 acid, polyglycolic acid, polylactic acid/polyglycolic acid copolymers and combinations
4 thereof, said polymer having a molecular weight of about 250 to about 7000 daltons.

1 **45.** The method of introducing a nucleic acid into a cell of claim 43,
2 wherein Y has at least 4 positive charges at a selected pH.

1 **46.** The method of introducing a nucleic acid into a cell of claim 43,
2 wherein Y is a member selected from the group consisting of lysine, arginine, asparagine,
3 glutamine, derivatives thereof and combinations thereof.

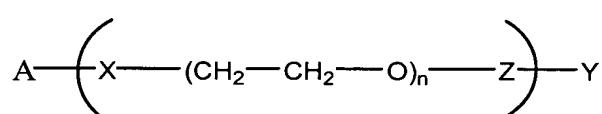
1 **47.** The method of introducing a nucleic acid into a cell of claim 43,
2 wherein A is a member selected from the group consisting of a diacylglycerolyl moiety, a
3 dialkylglycerolyl moiety, a N-N-dialkylamino moiety, a 1,2-diacyloxy-3-aminopropane
4 moiety and a 1,2-dialkyl-3-aminopropane moiety.

1 **48.** The method of introducing a nucleic acid into a cell of claim 43,
2 wherein W is PEG.

1 **49.** The method of introducing a nucleic acid into a cell of claim 43,
2 wherein W is a polyamide polymer.

1 **50.** The method of introducing a nucleic acid into a cell of claim 43,
2 wherein W has a molecular weight of about 250 to about 2000 daltons.

1 **51.** The method of introducing a nucleic acid into a cell of claim 48,
2 having the general structure of Formula II:



3 **II**

4 wherein

5 X is a member selected from the group consisting of a single bond or a
6 functional group covalently attaching said lipid to at least one ethylene oxide unit;

7 Z is a member selected from the group consisting of a single bond or a
8 functional group covalently attaching said at least one ethylene oxide unit to a cationic group;
9 and

10 n is an integer having a value of between about 6 to about 50.

1 **52.** The method of introducing a nucleic acid into a cell of claim **51**,
2 wherein

3 X is a member selected from the group consisting of a single bond,
4 phosphatidylethanolamino, phosphatidylethanolamido, phosphoro, phospho,
5 phosphoethanolamino, phosphoethanolamido, carbonyl, carbamate, carboxyl, carbonate,
6 amido, thioamido, oxygen, sulfur and NR, wherein R is a hydrogen or alkyl group.

1 **53.** The method of introducing a nucleic acid into a cell of claim **51**,
2 wherein

3 Z is a member selected from the group consisting of a single bond,
4 phosphatidylethanolamino, phosphatidylethanolamido, phosphoro, phospho,
5 phosphoethanolamino, phosphoethanolamido, carbonyl, carbamate, carboxyl, carbonate,
6 amido, thioamido, oxygen, sulfur and NR, wherein R is a hydrogen or alkyl group.

1 **54.** The method of introducing a nucleic acid into a cell of claim **51**,
2 wherein

3 A is a diacylglycerolyl moiety;

4 X is phosphoethanolamido;

5 Z is NR, wherein R is a hydrogen atom; and

6 Y is a member selected from the group consisting of about 1 to about 10 basic
7 amino acids or derivatives thereof.

1 **55.** The method of introducing a nucleic acid into a cell of claim **54**,
2 wherein

3 A is a diacylglycerolyl moiety having 2 fatty acyl chains, wherein each acyl
4 chain is independently between 2 and 30 carbons in length and is either saturated or has
5 varying degrees of saturation.

1 **56.** The method of introducing a nucleic acid into a cell of claim **54**,

2 wherein

3 Y is a member selected from the group consisting of lysine, arginine,
4 asparagine, glutamine, derivatives thereof and combinations thereof.

1 **57.** The method of introducing a nucleic acid into a cell of claim **54**,

2 wherein

3 A is a diacylglycerol moiety having 2 fatty acyl chains, wherein each acyl
4 chain is a saturated C-18 carbon chain; and

5 Y is a cationic group having 4 lysine residues or derivatives thereof.

1 **58.** The method of introducing a nucleic acid into a cell of claim **33**,

2 wherein said conjugated lipid that inhibits aggregation of particles is a PEG-lipid.

1 **59.** The method of introducing a nucleic acid into a cell of claim **58**,

2 wherein said PEG-lipid is PEG-ceramide.

1 **60.** The method of introducing a nucleic acid into a cell of claim **59**,

2 wherein the ceramide of said PEG-ceramide comprises a fatty acid group having about 8 to
3 about 20 carbon atoms.

1 **61.** The method of introducing a nucleic acid into a cell of claim **59**,

2 wherein said PEG-lipid is PEG-phosphatidylethanolamine.

1 **62.** The method of introducing a nucleic acid into a cell of claim **33**,

2 wherein said conjugated lipid that inhibits aggregation of particles is an ATTA-lipid.

1 **63.** The method of introducing a nucleic acid into a cell of claim **33**,

2 wherein said nucleic acid is selected from the group consisting of a plasmid, an antisense
3 oligonucleotide, and a ribozyme.

1 **64.** A method for inducing H_{II} phase structure in a lipid bilayer, said

2 method comprising: contacting said lipid bilayer with an endosomal membrane destabilizer,
3 thereby inducing H_{II} phase structure in a lipid bilayer.

1 **65.** The method for inducing H_{II} phase structure of claim **64**, wherein said

2 lipid bilayer comprises DOPC:DOPE:DOPS:Chol.

1 **66.** The method for inducing H_{II} phase structure of claim 64, wherein said
2 endosomal membrane destabilizer is Ca⁺⁺ ion.

1 **67.** The method for inducing H_{II} phase structure of claim 66, wherein Ca⁺⁺
2 ion acts in concert with low levels of the cationic lipid to trigger H_{II} phase formation.

1 **68.** Use of nucleic acid-lipid particle composition for introducing a nucleic
2 acid into a cell, said particle comprising: a cationic lipid, a conjugated lipid that inhibits
3 aggregation of particles, a nucleic acid and an endosomal membrane destabilizer.